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THE 3D STRUCTURES OF SOME DIARRHEAL CAUSING BACTERIAL TOXINS

ANNUAL REPORT

MARTIN SAX

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Veterans Administration Medical Center
University Drive C
Pittsburgh, Pennsylvania 15240

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(1) Statement of the Problem under Study. The determination of the crystal and molecular structure of diarrheal causing enterotoxins produced by S. aureus is the overall goal of this research effort. Obtaining the details of the 3D structures of the toxins is crucial for understanding the molecular mechanisms of the physiological processes which they induce in a host when infection occurs.

(2) Background and Review of Appropriate Literature and/or Earlier Report. The biochemical, biological, mitogenic and antigenic properties and activities of S. aureus toxins and enterotoxins have been studied extensively during the last two decades. The results have been reviewed recently in several chapters of volume 4 of the "Handbook of Natural Toxins" (1). In recent years intense scientific interest in S. aureus enterotoxins has resurfaced because of their ability to stimulate T cell proliferation. The answer to the question of how this happens is the immediate impetus for the spate of research activity which has revealed some fasciniating aspects of the T cell stimulation process. For instance, before T cell proliferation can be affected, the enterotoxin molecule must form a complex with a class II MHC molecule. The binary complex then must combine with the appropriate T cell receptor to yield a ternary complex which initiates T cell proliferation (2-7). Class II molecules apparently contain a unique binding site for the various S. aureus enterotoxins (5-7).

The positions of the binding sites involved in complex formation has been a topic of interest (6,7). However, the issues remain unresolved to the best of our knowledge. It is unequivocal however that the processing of enterotoxin by the class II molecules, such as occurs with normal antigens to yield polypeptide fragments for T cell presentation, is not a requirement for presenting an SEB molecule to the T cell receptor. The S. aureus enterotoxins furthermore differ from ordinary antigens in that they are much more potent T cell stimulators (7). They have much greater affinity for MHC class II molecules than do ordinary antigens. They have been called 'superantigens' because of their extraordinary potency in stimulating the immune system. Crystallographic work now in progress in this field consists of our analysis of the crystal structure of SEB (8).

(3) Rationale Used in Current Study. In order to understand the sterochemical mechanism of action of a protein molecule with confidence, knowledge of its 3D structure is essential. Presently this information is unavailable for S. aureus enterotoxins. When it becomes known, the problem then will be to relate the structural features to the processes involved, as for example the binding sites of the MHC class II molecule and of the T cell receptor. The connection between the structure and function can be made by observing the effects of point mutations on the activity of the enterotoxin, but in addition a priori knowledge of the crystal structure will be a valuable if not indispensible guide for designing the mutation experiments.

The 3D structure of SEB will be useful for modeling the binding to MHC class II molecules even in the absence of data from mutation experiments, since we already have available a proposed model of a class II molecule which is based upon the known 3D structure of a class I molecule (8,9). Comparing this model with the 3D structure of SEB will suggest likely sites where the class II molecule binds on the SEB molecular surface.

Since SEA, SEB and TSST (toxic shock syndrome toxin) have been shown to bind to the same site on the MHC class II molecule, HLA-DR (6) and since these toxins stimulate T cell proliferation (albeit different T cell populations), it is very plausible that their activities are variations of a common three

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dimensional structural motif. Accordingly, the structure function correlations found for SEB ought to possess general features applicable to classes of staphylococcal enterotoxins.

(4)Experimental Methods. We are applying standard crystallographic methods for solving protein structures to the determination of the crystal structure of SEB. The x-ray data are collected on a Xentronics area detector. The methods of multiple isomorphous replacement supplemented with anomalous dispersion data and with solvent leveling techniques are applied to the solution of the phase problem. Electron density maps are computed with these phases. The polypeptide chain is traced and the side chains are fitted to the density. The model is verified by refinement and by demonstrating that it is chemically and physically reasonable.

(5)Results. SEB crystallizes in two different forms:

Form I: a= 45.31 b= 70.61 c= 78.12A Space group = $P2_12_12_1$
Form II: a= 47.48 b= 68.30 c= 79.40A Space group = $P2_12_12_1$

The preliminary crystal data for form II have been published along with the crystallization procedure (10).

Intensity data were collected on our Xentronics area detector from a number of heavy atom derivatives. A 3.5A MIR electron density map was computed from a platinum and a mercury derivative. The mean figure of merit after applying solvent leveling was 0.8. The map was displayed on transparent sheets and analyzed. The molecular boundary was clear, but there were breaks in the polypeptide chain which obscured the fold in many places although several large stretches of contiguous electron density were consistent with expectations for a main chain.

A second electron density map was then computed with x-ray intensities from two platinum and four mercury derivatives but this time the anomalous scattering data were included. A 3.0A phase extended and solvent leveled map with a figure of merit of 0.88 clearly revealed two domains of unequal size, at least two strands of beta sheet, and a helix of 3 1/2 to 4 turns. We now are interpreting the map while continuing to search for more derivatives.

Besides the crystal structure analysis of SEB, we have been working on the production of other staphylococcal enterotoxins. We have harvested SEC1. The protein has been purified for use in crystallization experiments. Other SEs and S. aureus derived toxins, which stimulate T cell proliferation, will be produced and purified for use in crystal growth experiments.

(6)Discussion and Conclusions. The incorporation of data from additional heavy atom derivatives and the inclusion of anomalous dispersion data improved the electron density map markedly. The appearance of the two domain structure, which had been predicted previously from biochemical studies, makes us confident of the results obtained in our analysis. It encourages us to continue to pursue measures to improve the map.

The short alpha helix lying next to a segment of beta sheet resembles strikingly the structural motif in an MHC class I molecule binding site (8) which also is believed to be present in class II molecules (9). This further reinforces our feeling that SEB map is biologically and biochemically reasonable.

Although much remains to be done, a large step forward has been taken within the last year toward solving this stubborn and difficult crystallographic problem.

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